Mitogen-Activated Protein Kinase Pathways Mediated by ERK, JNK, and p38 Protein Kinases

Gary L. Johnson* and Razvan Lapadat

Multicellular organisms have three well-characterized subfamilies of mitogen-activated protein kinases (MAPKs) that control a vast array of physiological processes. These enzymes are regulated by a characteristic phosphorelay system in which a series of three protein kinases phosphorylate and activate one another. The extracellular signal–regulated kinases (ERKs) function in the control of cell division, and inhibitors of these enzymes are being explored as anticancer agents. The c-Jun amino-terminal kinases (JNKs) are critical regulators of transcription, and JNK inhibitors may be effective in control of rheumatoid arthritis. The p38 MAPKs are activated by inflammatory cytokines and environmental stresses and may contribute to diseases like asthma and autoimmunity.

Protein kinases are enzymes that covalently attach phosphate to the side chain of either serine, threonine, or tyrosine of specific proteins inside cells. Such phosphorylation of proteins can control their enzymatic activity, their interaction with other proteins and molecules, their location in the cell, and their propensity for degradation by proteases. Mitogen-activated protein kinases (MAPKs) compose a family of protein kinases whose function and regulation have been conserved during evolution from unicellular organisms such as brewers' yeast to complex organisms including humans (*1*). MAPKs phosphorylate specific serines and threonines of target protein substrates and regulate cellular activities ranging from gene expression, mitosis, movement, metabolism, and programmed death. Because of the many important cellular functions controlled by MAPKs, they have been studied extensively to define their roles in physiology and human disease. MAPK-catalyzed phosphorylation of substrate proteins functions as a switch to turn on or off the activity of the substrate protein. Substrates include other protein kinases, phospholipases, transcription factors, and cytoskeletal proteins. Protein phosphatases remove the phosphates that were transferred to the protein substrate by the MAPK. In this manner, the action of MAPKs and protein phosphatases reciprocally and rapidly alter the behavior of cells as they respond to changes in their environment.

MAPKs are part of a phosphorelay system composed of three sequentially activated kinases, and, like their substrates, MAPKs are regulated by phosphorylation (Fig. 1) (*2*). MAPKs serve as phosphorylation substrates for MAPK kinases (MKKs). MKK-catalyzed phosphorylation activates the MAPK and increases its activity in catalyzing the phosphorylation of its own substrates. MAPK phosphatases reverse the phosphorylation and return the MAPK to an inactive state. MKKs are highly selective in phosphorylating specific MAPKs. MAPK kinase kinases (MKKKs) are the third component of the phosphorelay system. MKKKs phosphorylate and activate specific MKKs. MKKKs have distinct motifs in their sequences that selectively confer their activation in response to different stimuli. Cells receive many different stimuli from their environment that influence their metabolic rate, interaction with other cells, survival and proliferative potential, and other cellular processes involved in homeostasis and health of the organism. One outcome of having many different MKKKs is that they can be matched with specific MKK-MAPK cassettes, such that cells can respond to different stimuli with the activation of a specific MAPK pathway.

In multicellular organisms, there are three well-characterized subfamilies of MAPKs. These MAPKs include the extracellular signal–regulated kinases, ERK1 and ERK2 (*3*); the c-Jun NH_2-terminal kinases, JNK 1, JNK 2, and JNK 3 (*4*); and the four p38 enzymes, p38α, p38β, p38γ, and p38δ (*5*). A fourth MAPK, ERK5, is a relatively recently identified MAPK and is being studied intensely (*6*). Additional protein kinases identified biochemically or during the sequencing of the human and mouse genomes may function as MAPKs but are still not well characterized. ERK7 is a possible MAPK candidate in this category (*7*).

ERK1 and ERK2

ERK1 and ERK2 are widely expressed and are involved in the regulation of meiosis, mitosis, and postmitotic functions in differentiated cells. Many different stimuli, including growth factors, cytokines, virus infection, ligands for heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors, transforming agents, and carcinogens, activate the ERK1 and ERK2 pathways. ERKs 1 and 2 are both components of a three-kinase phosphorelay module that includes the MKKK c-Raf1, B-Raf, or A-Raf, which can be activated by the proto-oncogene *Ras*. Mutations that convert Ras to an activated oncogene are common oncogenic mutations in many human tumors. Oncogenic Ras persistently activates the ERK1 and

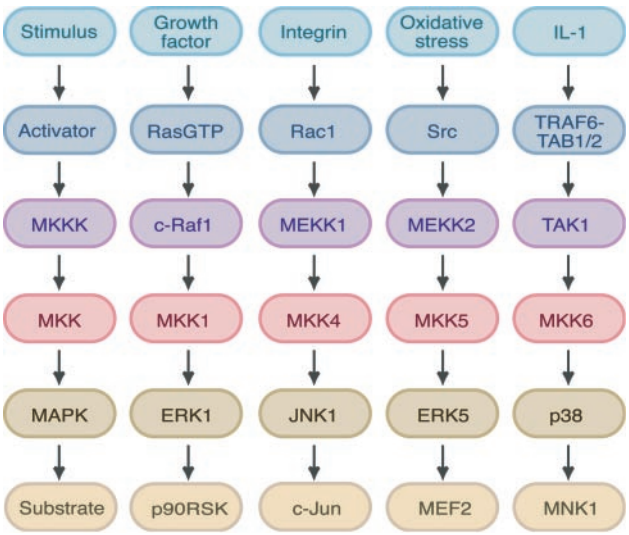

Fig. 1. MAPK phosphorelay systems. GTP, guanosine triphosphate; p90RSK, 90-kD ribosomal protein S6 kinase, Src, an oncogenic tyrosine kinase; MEF2, myocyte enhancer factor 2; IL-1, interleukin 1; TRAF6, tumor necrosis factor receptor–associated factor 6; TAK1, transforming growth factor-β–activated protein kinase 1; and MNK1, MAPK-interacting kinase 1. The modules shown are representative of pathway connections for the respective MAPK phosphorelay systems. There are multiple component MKKKs, MKKs, and MAPKs for each system. For example, there are three Raf proteins (c-Raf1, B-Raf, A-Raf), two MKKs (MKK1 and MKK2), and two ERKs (ERK1 and ERK2) that can compose MAPK phosphorelay systems responsive to growth factors. The ERK, JNK, and p39 pathways in the STKE Connections Map demonstrate the potential complexity of these systems. Our understanding of the connections within the MAPK systems is incomplete and often controversial and continues to be defined in different cell types.

Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262, USA.

*To whom correspondence should be addressed. E-mail: gary.johnson@uchsc.edu

ERK2 pathways, which contributes to the increased proliferative rate of tumor cells. For this reason, inhibitors of the ERK pathways are entering clinical trials as potential anticancer agents. In differentiated cells, ERKs have different roles and are involved in responses such as learning and memory in the central nervous system.

JNK1, JNK2, and JNK3

The JNKs were isolated and characterized as stress-activated protein kinases on the basis of their activation in response to inhibition of protein synthesis (*8*). The JNKs were then discovered to bind and phosphorylate the DNA binding protein c-Jun and increase its transcriptional activity. c-Jun is a component of the AP-1 transcription complex, which is an important regulator of gene expression. AP-1 contributes to the control of many cytokine genes and is activated in response to environmental stress, radiation, and growth factors — all stimuli that activate JNKs. Regulation of the JNK pathway is extremely complex and is influenced by many MKKKs. As depicted in the STKE JNK Pathway Connections Map, there are 13 MKKKs that regulate the JNKs. This diversity of MKKKs allows a wide range of stimuli to activate this MAPK pathway. JNKs are important in controlling programmed cell death or apoptosis (*9*). The inhibition of JNKs enhances chemotherapy-induced inhibition of tumor cell growth, suggesting that JNKs may provide a molecular target for the treatment of cancer. JNK inhibitors have also shown promise in animal models for the treatment of rheumatoid arthritis (*10*). The pharmaceutical industry is bringing JNK inhibitors into clinical trials for both diseases.

p38 Kinases

There are four p38 kinases: α, β, γ, and δ. The p38α enzyme is the most well characterized and is expressed in most cell types. The p38 kinases were first defined in a screen for drugs inhibiting tumor necrosis factor α–mediated inflammatory responses (*11*). The p38 MAPKs regulate the expression of many cytokines. p38 is activated in immune cells by inflammatory cytokines and has an important role in activation of the immune response. p38 MAPKs are activated by many other stimuli, including hormones, ligands for G protein–coupled receptors, and stresses such as osmotic shock and heat shock. Because the p38 MAPKs are key regulators of inflammatory cytokine expression, they appear to be involved in human diseases such as asthma and autoimmunity.

Recently, a major paradigm shift for MAPK regulation was developed for p38α. The p38α enzyme is activated by the protein TAB1 (*12*), but TAB1 is not a MKK. Rather, TAB1 appears to be an adaptor or scaffolding protein and has no known catalytic activity. This is the first demonstration that another mechanism exists for the regulation of MAPKs in addition to the MKKK-MKK-MAPK regulatory module. This important observation indicates that other adaptor proteins should be scrutinized for potential roles in regulating MAPK activity.

The importance of MAPKs in controlling cellular responses to the environment and in regulating gene expression, cell growth, and apoptosis has made them a priority for research related to many human diseases. The ERK, JNK, and p38 pathways are all molecular targets for drug development, and inhibitors of MAPKs will undoubtedly be one of the next group of drugs developed for the treatment of human disease (*13*).

References and Notes

1. C. Widmann *et al.*, *Physiol. Rev.* **79**, 143 (1999).
2. L. B. Ray, T. W. Sturgill, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3753 (1988).
3. G. L. Johnson, R. Lapadat, ERK Pathway, *Science's STKE* (Connections Map, as seen November 2002), http://stke.sciencemag.org/cgi/cm/stkecm;CMP_10705.
4. _____, JNK Pathway, *Science's STKE* (Connections Map, as seen November 2002), http://stke.sciencemag.org/cgi/cm/stkecm;CMP_10827.
5. _____, p38 Pathway, *Science's STKE* (Connections Map, as seen November 2002), http://stke.sciencemag.org/cgi/cm/stkecm;CMP_10958.
6. G. Zhou *et al.*, *Biol. Chem.* **270**, 12665 (1995).
7. M. K. Abe *et al.*, *Mol. Cell. Biol.* **19**, 1301 (1999).
8. J. M. Kyriakis *et al.*, *Nature* **369**, 156 (1994).
9. C. Tournier *et al.*, *Science* **288**, 870 (2000).
10. Z. Han *et al.*, *J. Clin. Invest.* **108**, 73 (2001).
11. J. C. Lee *et al.*, *Nature* **372**, 739 (1994).
12. B. Ge *et al.*, *Science* **295**, 1291 (2002).
13. J. M. English, M. H. Cobb, *Trends Pharmacol. Sci.* **23**, 40 (2002).

REVIEW

The Protein Kinase Complement of the Human Genome

G. Manning,[1]* D. B. Whyte,[1] R. Martinez,[1] T. Hunter,[2] S. Sudarsanam[1,3]

We have catalogued the protein kinase complement of the human genome (the "kinome") using public and proprietary genomic, complementary DNA, and expressed sequence tag (EST) sequences. This provides a starting point for comprehensive analysis of protein phosphorylation in normal and disease states, as well as a detailed view of the current state of human genome analysis through a focus on one large gene family. We identify 518 putative protein kinase genes, of which 71 have not previously been reported or described as kinases, and we extend or correct the protein sequences of 56 more kinases. New genes include members of well-studied families as well as previously unidentified families, some of which are conserved in model organisms. Classification and comparison with model organism kinomes identified orthologous groups and highlighted expansions specific to human and other lineages. We also identified 106 protein kinase pseudogenes. Chromosomal mapping revealed several small clusters of kinase genes and revealed that 244 kinases map to disease loci or cancer amplicons.

Ever since the discovery nearly 50 years ago that reversible phosphorylation regulates the activity of glycogen phosphorylase, there has been intense interest in the role of protein phosphorylation in regulating protein function. With the advent of DNA cloning and sequencing in the mid-1970s, it rapidly became clear that a large family of eukaryotic protein kinases exists, and the burgeoning numbers of protein kinases led to the speculation that a vertebrate genome might encode as many as 1001 protein kinases (*1*). The near-completion of the human genome sequence now allows the identification of almost all human protein kinases. The total (518) is about half that predicted 15 years ago, but it is still a strikingly large number, constituting about 1.7% of all human genes.

Protein kinases mediate most of the signal transduction in eukaryotic cells; by modification of substrate activity, protein kinases also control many other cellular processes, including metabolism, transcription, cell cycle progression, cytoskeletal rearrangement and cell movement, apoptosis, and differentiation. Protein phosphorylation also plays a critical

[1]SUGEN Inc., 230 East Grand Avenue, South San Francisco, CA 94080, USA. [2]Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. [3]Genomics and Biotechnology, Pharmacia Corporation, 230 East Grand Avenue, South San Francisco, CA 94080, USA.

*To whom correspondence should be addressed. E-mail: gerard-manning@sugen.com